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Protector turns predator

Autophagic death via selective degradation of KRAS

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Therapy-induced autophagy is recognized as a critical determinant of treatment outcome in cancer patients, primarily as a factor underlying drug resistance. However, recent investigations point toward a context-dependent, death-inducing role for autophagy, the mechanism of which remains largely unknown. Our recent study provides evidence that autophagy can directly mediate cell killing in multiple tumor cell types by facilitating degradation of KRAS/K-Ras, a key survival protein. These findings have broad implications for strategies employing autophagy modulation to target tumor cells.

Traditionally, autophagy, a form of 'self eating,' has been well-accepted to be an integral component of the cell's prosurvival machinery. With functions ranging from routine 'garbage disposal' to adaptive responses involved in warding off a microbial attack or other stresses, autophagy serves to buffer a cell against a wide array of damaging stimuli. However, attempts to enhance therapy-induced tumor cell cytotoxicity by attenuating prosurvival autophagy have yielded mixed results, thereby questioning the general acceptance of autophagy as strictly a survival mechanism. This has also led to the seemingly paradoxical concept of "autophagic death," which is defined as "death caused by the autophagy pathway." It is now widely accepted that, depending on biological and cellular context, therapy-induced autophagy can either contribute to drug resistance or enhance tumor cell killing. The mechanisms by which therapy-induced autophagy might trigger cell killing have remained largely unknown.

Recently, we demonstrated that drug-induced autophagy can directly participate in cell killing via the degradation of KRAS, a key survival protein. This study was performed in the context of 4-hydroxy tamoxifen (OHT)-induced autophagy. It was aimed at identifying estrogen receptor (ESR)-independent cell death mechanisms in tumor cells to explain the efficacy of OHT in multiple ESR-negative tumors. We observed that OHT triggers activation of effector caspases, but broad caspase inhibition has no impact on OHT-induced death, thereby indicating a role for nonapoptotic mechanisms of death. However, OHT also induces a robust autophagic response, which when blocked, attenuates OHT-induced cytotoxicity, leading us to infer a prodeath role for autophagy. Since the basic function of autophagy is to facilitate turnover of long-lived proteins, we hypothesized that autophagy may disrupt the balance between prosurvival and prodeath proteins resulting in cell death. A survey of the list of genes whose loss mediates sensitivity to OHT-induced death reveals a prosurvival role for the RAS isoform KRAS. In addition, direct knockdown of KRAS levels supports its role in mediating resistance to OHT-induced death. An assessment of the levels of KRAS following OHT treatment revealed a decrease in levels that is not accompanied by changes at the transcriptional or translational level, but is

blocked using pharmacological and genetic means to inhibit autophagy induction. We assessed the functional impact of decreased KRAS levels on downstream effector pathways and observed a time-dependent decrease in levels of activated MAP kinases (MAPKs) such as JNK and MAPK1/3. This observation is consistent with previous studies indicating that the prosurvival effects of KRAS activation are mediated, in part, by MAPK signaling. Collectively, these observations led us to conclude that OHT triggers autophagy-mediated death in MPNST cells through increased KRAS degradation, potentially, due to decreased MAPK signaling.

These findings offer new insights into a previously unexplored mechanism of autophagic death, but also give rise to several questions. It remains to be determined whether other autophagy inducers can mimic the functional consequences of OHT-induced autophagy or if additional OHT targets poise KRAS for autophagic degradation. We propose that OHT might prime KRAS for autophagic degradation by disrupting its stability at the plasma membrane. KRAS retains its association with the plasma membrane through its polybasic region. Disruption of this interaction via modulation of factors such as intracellular calcium levels and protein kinase C (PRKC), a known OHT target, can potentially mark KRAS for degradation. Two lines of evidence support this hypothesis. First, we observed that direct PRKC inhibition also accelerates KRAS degradation. In addition, levels of epidermal growth factor receptor, a protein that is internalized from the plasma membrane and compartmentalized along with KRAS en route to degradation, were also decreased. This finding suggests that OHT might trigger changes in membrane dynamics, thereby altering the stability of membrane bound proteins and directing them for degradation. While our results suggest a potential role for PRKC, we recognize that these findings are correlative and there could be additional upstream stimuli mediating OHT effects. However, since PRKC has known phosphorylation sites on KRAS that modulate its stability on the plasma membrane, nonphosphorylatable KRAS mutants can be generated to specifically assess the impact of PRKC phosphorylation on KRAS degradation. Furthermore, it would be of interest to assess if known KRAS mutations can either alter the ability of PRKC to interact with KRAS or can interfere with its ability to be targeted via the autophagy pathway due to a lack of association with adaptor proteins. Our initial results with cell lines harboring a mutant KRAS certainly suggest a propensity for the G13D mutant to resist degradation. However, this hypothesis remains to be directly tested since additional mutations in the tested cell lines might lead to the block in OHT-triggered KRAS degradation.

Overall, this study reveals a novel strategy for potentially targeting the KRAS pathway that underlies drug resistance in several aggressive malignancies. Previous attempts to target KRAS have focused on post-translational modifications and downstream signaling effectors. However, a clear understanding of strategies to facilitate KRAS degradation might aid in effective targeting of tumors. These findings also indicate that the cytoprotective vs. cytotoxic effects of autophagy in varying scenarios might result from the identity of the proteins being targeted for degradation. It will be interesting to determine if additional autophagy targets can be directly implicated in regulating treatment outcomes and if this can be exploited in clinical settings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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